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(54) Title: BIODEGRADATION OF OIL SLUDGE

(57) Abstract

A method for the biodegradation of an oil–based sludge comprising a mixture of petroleum hydrocarbons is disclosed. The method comprises forming an aqueous solution in a reactor of an oil–in–water emulsion of the oil–based sludge, bacterial culture and nutrients for the bacterial culture, the bacterial culture having the ability to grow on petroleum hydrocarbons as sole carbon source and having been isolated from a hydrocarbon contaminated soil or hydrocarbon–containing sludge or other environments rich in hydrocarbon degrading bacteria, maintaining the aqueous solution under aerobic conditions in the reactor at a temperature of at least 10 °C for a period of time sufficient to reduce the amount of hydrocarbon by at least 25 %, and discharging aqueous solution having a reduced amount of hydrocarbons from the reactor. The method may be used on sludge containing aromatics, resins and asphaltenes.

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# BIODEGRADATION OF OIL SLUDGE

The present invention is directed to the treatment of oil sludges, and in particular to biodegradation of oil sludges to environmentally-acceptable products. As such, the present invention is directed to the treatment of compositions with high sludge/total petroleum hydrocarbon concentrations, examples of which are oil refinery sludges, tank-bottom sludges from oil storage tanks or tankers, sludges from residues at oil wells, so-called slop oil or treater emulsions, oil sludges from processing of solids containing oil wastes including centrifuged sludges, clay fines, and drilling mud residues. In contrast to waste water treatment processes utilizing low total petroleum hydrocarbon concentrations or processes for the production of single cell protein, biomass or bacterial cells.

Biodegradation of crude oil materials has primarily been directed to the clean up i.e. bioremediation, of oil-contaminated soils and shorelines, as a result of on-land oil spills from, for example, underground storage tanks, or from oil tankers at sea. Such bioremediation of hydrocarbons generally involves creation of conditions in the soil or on the shoreline that promote growth of microorganisms using the petroleum hydrocarbons, facilitating conversion of the hydrocarbons to biomass and/or their degradation, ultimately to carbon dioxide and water. The hydrocarbons are the source of carbon for microbial growth, although it may be necessary to add other ingredients, especially nitrogen and phosphorus, as fertilizers. Microorganisms also require a range of inorganic ions for growth, but such ions are generally present in adequate quantities in the soil that is being treated.

Bioremediation processes generally utilize aerobic microorganisms that require aeration/oxygenation by maximizing contact of the contaminated material with atmospheric oxygen through procedures of soil tilling or by aerating using positive or negative pressure air pumping systems.

The general hierarchy of microbial activity in crude oil is understood to be

aliphatics>aromatics>resins>asphaltenes.

Thus, aromatic and high molecular weight hydrocarbons are more difficult to degrade, compared to the lower alkanes.

Liquid-solid treatment systems have also been used to degrade petroleum hydrocarbons. However, long degradation treatment periods were encountered, e.g. 50-100 days. Land treatment of waste crude oils and refinery oil sludges has been used for many years as a method of disposal of oil and sludge. Microbial growth and biodegradation rates tend to be suboptimal in land farming processes and the process is not easily controlled. In addition the process is influenced by soil composition, weather and temperature, as well as the methods used for tilling in the land farming process. For large refineries, large areas of land have to be committed to such a process, and moreover the first step in the process involves contamination of the soil with the oils to be degraded.

U.S. 3 899 376 discloses a single or multi-tank system that is primarily directed to waste water treatment. The process utilizes a particular bacterial strain from a culture collection for the bioremediation process.

U.S. 5 364 789 discloses a microbial cleaner comprising at least one hydrocarbon-ingesting microbe strain and a biocatalyst that transforms hydrocarbons into non-toxic substances. The biocatalyst includes a non-ionic surfactant, a chlorine-absorbing salt, at least one microbe nutrient and water. It is stated that the cleaner may be used in virtually any situation requiring the removal of hydrocarbons, including cleaning contaminated soil and treating oil spills on soil and water.

A method for the biodegradation of a petroleum hydrocarbon sludge fraction has now been found, such method using a reactor.

Accordingly, an aspect of the present invention provides a method for the biodegradation of an oil-based sludge, said oil-based sludge comprising a mixture of petroleum hydrocarbons, said method comprising the steps of:

(a) forming an aqueous solution in a reactor of an oil-in-water emulsion, bacterial culture and nutrients for said bacterial culture,

said oil-in-water emulsion being an emulsion of said oil-based sludge in water,

said bacterial culture having the ability to grow on petroleum hydrocarbons as sole carbon source and having been isolated from a hydrocarbon contaminated soil or hydrocarbon-containing sludge or other environments rich in hydrocarbon degrading bacteria, by microbial enrichment techniques using hydrocarbons in the selection medium,

said reactor containing up to 50% by volume of said hydrocarbons;

(b) maintaining said aqueous solution under aerobic conditions in the reactor at a temperature of at least 10°C for a period of time sufficient to reduce the amount of hydrocarbon by at least 25%, and at a pH conductive for promotion of bacterial growth and hydrocarbon degradation; and

(c) discharging aqueous solution having a reduced amount of said hydrocarbons from said reactor.

In a preferred embodiment of the present invention, the nutrients comprise bioavailable nitrogen, phosphorous and potassium compounds, especially in which the nitrogen compound is an ammonium ion, nitrate or organic nitrogen, and the phosphorus is phosphate.

In another embodiment, the reactor contains about 5-50% by volume of said petroleum hydrocarbons, especially about 10-30% by volume of said petroleum hydrocarbons. The oil-based sludge preferably contains hexane-extractable hydrocarbons in an amount in the range of up to 500 000 ppm, especially in the range of 65 000-250 000 ppm.

In yet another embodiment, the nutrients are in proportions corresponding to relative proportions in bacterial cells, and supplied at concentrations which promote high levels of bacterial growth and high rates of hydrocarbon degradation.

In further embodiments, the petroleum hydrocarbons consist of mixtures of saturated hydrocarbons, aromatic hydrocarbons, hydrocarbon resins and asphaltenes, especially petroleum hydrocarbons obtained from petroleum refinery sludge, from the bottom of a storage tank for oil, from an on-land well head or from the washing of a hold in a tanker.

In other embodiments, the amount of nitrogen required to support the process is in the range of 50-1000 ppm, and preferably in the range of 300-700 ppm, and the minimum amount of phosphate is in the range of 10-200 ppm and preferably 50-150 ppm.

In additional embodiments, the aqueous solution contains a surfactant, more especially a non-ionic or an anionic surfactant. The surfactant is in an amount sufficient to form said oil-in-water emulsion, especially in which the amount of surfactant is less than 2500 ppm and preferably less than 1500 ppm. It is preferred that the ratio of the amount of petroleum hydrocarbon to surfactant be at least 40:1.

The method of the present invention relates to the biodegradation of an oil-based sludge. The oil-based sludge comprises a mixture of petroleum hydrocarbons and may include non-petroleum solid or liquid contaminants and water. The petroleum hydrocarbon mixture would normally comprise a mixture of aliphatic hydrocarbons, aromatic hydrocarbons, hydrocarbon resins and asphaltenes.

The present invention is particularly directed to the biodegradation of a mixture of the petroleum hydrocarbon from among the aliphatics, aromatics, resins and asphaltenes. Such mixtures of petroleum hydrocarbons may be obtained from a variety of sources. For instance, the mixture may be in form of a sludge obtained from a petroleum refinery. The sludge may also be obtained from the bottom of a storage tank that has been used for the storage of petroleum oil, with the sludge being obtained particularly when the storage tank is cleaned or drained. Alternatively, the mixture of hydrocarbons could be a

petroleum residue obtained from around an on-land well head, be an oil-containing clay fines material or be or from the cleaning of a hold of a tanker used for the transportation of petroleum products. The mixture of petroleum hydrocarbons, which is referred to herein as a sludge, may also be obtained from a variety of other sources. In each case, the sludge is characterized by having a substantial proportion of heavy end petroleum hydrocarbons which may require use of a solubilizing agent or surfactant to facilitate mixing and dispersal in water, as an oil-in-water emulsion.

The method of the present invention is carried out in a reactor. It is preferred that the reactor be a single stage reactor that is charged with the solution described herein, allowed to incubate for a period of time to reduce the amount of hydrocarbons within the aqueous solution, and then subsequently discharged from the reactor. Nonetheless, it is to be understood that the reactor could be in the form of a series of reactors in which the aqueous solution is passed from reactor to reactor before being finally discharged from the process for the biodegradation of the sludge.

In the method, an aqueous solution is fed to the reactor. The aqueous solution is comprised of an oil-in-water emulsion, bacterial culture and nutrients for the bacterial culture. The sludge is in the form of the oil-in-water emulsion.

The amount of petroleum hydrocarbons fed to the reactor is primarily governed by the formation of the oil-in-water emulsion. In particular, the aqueous solution may contain up to 50% by volume of petroleum hydrocarbons, depending on the particular hydrocarbons,

or higher if the petroleum hydrocarbons will permit formation of oil-in-water emulsions at higher loadings. In preferred embodiments, the reactor contains 5-50% by volume of the petroleum hydrocarbons, especially 10-30% by volume.

The oil-based sludge contains hexane-extractable hydrocarbons. In preferred embodiments, the amount of hexane-extractable hydrocarbons is up to 500 000 ppm, especially in the range of 65 000 - 250 000 ppm.

It would normally be necessary to incorporate a surfactant into the aqueous solution and to subject the aqueous solution to agitation in order to form the oil-in-water emulsion. The surfactant is preferably a non-ionic or an anionic surfactant, and is used in an amount sufficient to form the emulsion. Nonetheless, the amount of the surfactant is preferably less than 2500 and particularly less than 1500 ppm. In addition, the amount of surfactant, if added, is maintained at as low a level as is consistent with obtaining the oil-in-water emulsion. In particular, it is preferred that the ratio of petroleum hydrocarbon to surfactant be at least 40:1, and especially at least 60:1.

The aqueous solution also contains a bacterial culture. The bacterial culture used in the method of the present invention is a natural-occurring bacterial culture. Such a culture may be isolated from a hydrocarbon-contaminated soil or from hydrocarbons-containing sludge or from other environments, including soil or activated sludge, which may be rich in hydrocarbon-degrading bacteria, and inoculated in a basal medium, as described herein. The bacterial culture is selected by its ability to grow on petroleum hydrocarbons

as the predominant source of carbon in the basal medium.

Bacterial enrichment techniques for isolation of a bacterial culture capable of growing on hydrocarbons are well understood in the art. Typical techniques comprise adding a sample of soil, sludge or other material containing a large population of bacteria to an aqueous medium containing hydrocarbons as the only or predominant carbon source. Other chemical components including an inorganic nitrogen source, phosphorous and salts necessary to support bacterial growth are also added. Such a medium can be used to preferentially promote multiplication of hydrocarbon-degrading bacteria using standard aerobic microbial cultivation methods, including incubation in aerated microbial culture vessels. By transfer of a small amount of the resultant growth culture to further samples of the same medium and repeating the process one or more times, an efficient hydrocarbon degrading culture is selected. The culture can be maintained or stored using methods well known in the art.

In order to prepare a high density culture for use as an inoculum for sludge degradation, the maintained culture may be inoculated into an aqueous medium consisting of the nutrients described herein, supplemented with petroleum hydrocarbons and incubated in an aerated reactor or fermenter or other culture vessel. The preferred inoculum volume is 0.1-20% by volume of total culture volume, preferably 1-5% by volume. The preferred concentration of petroleum hydrocarbons used in this inoculum development medium is 0.5-5%, and can be obtained from various sources including petroleum sludges, crude oils or refined oils such as diesel oil.

A typical aeration rate of the inoculum reactor is 0.1-1.0 volumes of air per volume of medium per minute, with the culture incubated in the temperature range 20-37°C for 1-7 days, preferably at 27-33°C, at a pH generally maintained in the range 6.5-8.0, preferably in the range 7-7.5. The resultant bacterial culture maybe used to inoculate the reactor containing the sludge to be degraded, at a rate of 0.1-20% of total sludge volume, preferably 1-5%. Where a much larger volume of inoculum is required, the resultant inoculum may be transferred as an inoculum to a larger culture vessel and the culture development process repeated on the larger scale.

The aqueous solution fed to the reactor also contains nutrients for the bacterial culture. A wide variety of nutrients for the bacterial culture may be used, as will be understood by persons skilled in the art. Such nutrients will include nitrogen, phosphorus and potassium compounds, and would normally also include a variety of other ingredients. In particular, the nutrients comprise bioavailable nitrogen and phosphorus compounds. In embodiments, the amount of nitrogen is in the range of 50-1000 ppm and preferably 400-700 ppm, and the amount of phosphate is in the range of 10-200 ppm and preferably 50-150 ppm. In addition to nitrogen and phosphorus compounds, the nutrient also contains optimized concentrations of compounds other than nitrogen, phosphorus, carbon, oxygen and sodium, required to support bacterial growth and therefore it is normally necessary to add to the reactor one or more of magnesium, manganese, inorganic or organic sulphur, calcium, iron, copper, cobalt, zinc, boron and molybdenum. It will be appreciated that a guide for selection of the relative

amounts of nitrogen, phosphorus and other required nutrients is to relate their concentrations to the amounts of these components present in bacterial cells.

By providing an appropriate balance of nutrients and by adjustment of nutrient concentration, it is possible to achieve high levels of growth of hydrocarbon degrading bacteria and thus accelerated rates of hydrocarbon degradation. For example, Greasham (1993) "Biotechnology, a multivolume comprehensive treatise" (Eds, Rehm, H.J., *et al*) Vol. 3, p.131, VCH, Weinheim) has reported the typical non-carbon elemental composition of major bacterial components to be nitrogen 12.5%; phosphorus, 2.5%; potassium, 2.5%; sodium, 0.8%; sulphur, 0.6%; calcium, 0.6%; magnesium, 0.3%; copper, 0.02%; manganese, 0.01% and iron, 0.01%. Use of appropriate concentrations and ratios of nutrients tends to avoid a situation where growth is limited by depletion of one essential nutrient while all other nutrients may be present in excess.

The hydrocarbon provides the carbon source for growth; oxygen is obtained by aeration of the culture; sodium is provided in the form of caustic soda, required to adjust the pH. It is also understood that in some cases, some of these nutrient components may be present in sufficient quantities in some petroleum sludges or added water such that addition of selected nutrients may in some cases not be required. A disadvantage of relying on nutrients present as contaminants in sludge or water is that their concentrations may be variable, thus introducing inconsistencies into the process.

An example of a nutrient composition is as follows:

| | | |
|---|---|---|
| N | as $NH_4$,$NO_3$, or organic N | 500-700 ppm |
| P | as phosphate or related form | 100-120 ppm |
| K | | 50-90 ppm |
| Mg | | 10 ppm |
| Mn | | 1-4 ppm |
| S | as sulphate or organic sulphur | 15 ppm |
| Ca | | 8-12 ppm |
| Ferric Ion | | 1 ppm |
| Copper | | 0.5 ppm |
| Surfactant (nonionic or anionic) | | 1250 ppm |
| Co, Zn, B, Mo | | 5-10 ppb each |

The relative ratios of these nutrients are similar to the ratios typically found in the compositions of bacterial cells.

Other examples of nutrient compositions are given in the Examples herein below.

The aqueous solution in the reactor is maintained at a temperature of a least 10°C. Preferred temperatures are 15-37°C, and especially 20-33°C. The aqueous solution is maintained in the reactor for a period of time sufficient to reduce the amount of total petroleum hydrocarbon by at least 25%, especially by at least 50%. Typical times to effect the reduction in total petroleum hydrocarbon is 5-20 days, depending on the petroleum hydrocarbon being treated and the reactor conditions.

Subsequent to maintaining the aqueous solution at the predetermined temperature for a period of time, the aqueous solution is discharged from the reactor. The aqueous solution has a reduced amount of hydrocarbons,

including a reduced amount of the hydrocarbons from the group comprising the aromatics, resins and asphaltenes.

The present invention may be used for the biodegradation of sludges, as described herein. In particular, it may be used for biodegradation of a combination of hydrocarbon components from among the fractions: saturates, aromatics, resins and asphaltenes. It may also be used to preferentially degrade a proportion of the hydrocarbons, in a manner which causes the emulsion to break and facilitate separation of a water phase and a residual oil phase. The residual oil phase may be recovered for reuse. Alternatively, the oil phase may be recycled to the next reactor cycle with the water phase only being discharged from the reactor. The water phase contains high concentrations of hydrocarbon-degrading bacteria. Thus, the water phase may be used for processes including soil bioremediation processes, by direct spraying of the water on the contaminated soil. Alternatively, the bacteria maybe recovered from the water phase by known methods (filtration or centrifugation) and subsequently the bacteria may be applied in these other processes.

Where subsequent batches of sludge are to be degraded in the reactor, a portion of the degraded sludge amounting for example, to 1-20% of reactor volume, may be retained in the reactor following discharge, as an inoculum source for the next sludge batch.

In addition to the above described batch sludge degradation process, it is envisaged that the invention extends to fed-batch, continuous and semi-continuous reactor processes. In the fed-batch process, after the batch process has proceeded for some time, additional

sludge and/or nutrients/surfactant are added at one or more intervals and the process is allowed to continue. In continuous or semi-continuous processes, degraded sludge is removed from the reactor and replaced with undegraded sludge and nutrients/surfactants on a continuous basis or at intervals, respectively.

The invention is illustrated by the following examples. Unless stated to the contrary, all examples of the invention illustrated herein were conducted under non-sterile conditions. In addition, all biodegradation reactions exemplified herein used oil-in-water emulsions.

## EXAMPLE I

The basal medium used in this example contained (per L): $KH_2PO_4$, 1.0 g; $Na_2HPO_4$, 1.5 g; $MgSO_4.7H_2O$, 0.2 g; $Na_2CO_3$, 0.1 g; $CaCl_2.2H_2O$, 0.05 g; $FeSO_4$, 0.005 g; $MnSO_4$, 0.02 g; and trace metal solution, 3 ml. The trace metal solution contained (per L): $ZnCl_2.4H_2O$, 0.0144 g; $CoCl_2$, 0.012 g; $Na_2MoO_4.2H_2O$, 0.012 g; $CuSO_4.5H_2O$, 1.9 g; $H_3BO_4$, 0.05 g: and HCl, 35 ml. The initial pH of the nutrient was adjusted to 7.2.

A population of mixed bacterial culture was maintained in a cyclone fermenter with a working volume of one litre. Petroleum hydrocarbon-degrading bacteria were selected by their ability to grow on petroleum hydrocarbons as the sole carbon source in the basal medium described above. To initiate the selection of petroleum hydrocarbon-degrading bacterial culture, a mixed population of bacteria, isolated from hydrocarbon contaminated soil, was inoculated into basal medium supplemented with 2.0 g $NH_4Cl$/L and 1.0 g $NaNO_3$/L in the cyclone. Sludge A or B (60 g/L) was used as carbon

source; the sludges are describe below. It was found that the bacterial population reached $10^8$ to $10^{10}$ CFU/ml in one week. Thereafter, the culture was maintained by removing 10% by volume of the reactor and replacing with 10% by volume of fresh basal medium and sludge every day. Using this procedure, an actively growing culture was maintained.

Sludge samples were collected from different ponds or lagoons located at different oil refineries. TPH content (hexane extractable) was determined for each sludge. The composition of the different sludges is provided in Table 1.

**Table 1.**

| Sludge Source | Hexane Soluble(%) | Hexane insoluble(%) | Water (%) |
|---|---|---|---|
| Sludge A | 25 | 13 | 62 |
| Sludge B | 13 | 3 | 84 |
| Sludge C | 12 | 11 | 77 |
| Sludge D | 65 | 15 | 20 |
| Sludge E | 31 | 16 | 53 |
| Sludge F | 22 | 6 | 72 |
| Sludge G | 89 | 11 | 0 |

## EXAMPLE II

The nutrient medium used for biodegradation in this example consisted of the basal medium supplemented with 2.0 g urea/L and 1.0 g yeast extract/L.

Runs to determine the biodegradation of total petroleum hydrocarbons (TPH) with respect to incubation time were carried out in 250 ml Erlenmeyer flasks containing 10 ml of nutrient medium and 10 g of sludge, giving a final sludge concentration of 50% in the total flask contents. The flasks were inoculated with 0.6 ml

of actively growing mixed culture from the cyclone, maintained as described above, and incubated for 24 days at 25°C.

Residual TPH content was determined as follows. At different time intervals, whole flask contents were extracted with 40 ml of hexane and centrifuged at 10 000 rpm for 20 minutes. The hexane layer (top) was pipetted out and transferred to a pre-weighed vial. The hexane was allowed to evaporate in a fumehood and residual oil was weighed to determine total petroleum hydrocarbons (TPH).

The results are given in Table 2.

**Table 2.**

| Incubation Time (Days) | TPH degradation (%) |
|---|---|
| 6 | 32 |
| 14 | 37 |
| 18 | 47 |
| 24 | 48 |

It was found that over a period of 18 days, about 47% degradation of TPH occurred. No significant difference in degradation levels was obtained between 18 days and 24 days.

## EXAMPLE III

In order to investigate the effect of surfactant on TPH biodegradation, 5 different surfactants were tested at 0.25% concentration.

In each test, 10 ml of nutrient medium, 10 g of sludge oil and 0.5 ml of stock surfactant solution (10% aqueous) were placed in a 250 ml Erlenmeyer flask. The

contents of the flask were inoculated with 0.6 ml of actively growing culture from a cyclone fermenter and incubated on a rotary shaker (200 rpm) for 14 days at 25°C. Residual TPH content was determined after extraction with hexane.

The results are given in Table 3.

**Table 3.**

| Surfactant | TPH degradation (%) |
|---|---|
| None | 46 |
| Igepal™ CO-630 | 66 |
| Biosoft™ EN-600 | 63 |
| Sorbax™ PM030 | 42 |
| Witcomul™ 4078 | 41 |
| Marlipal™ O 13/120 | 45 |

All surfactants gave an oil-in-water emulsion. Out of 5 surfactants tested, 2 surfactants viz. Igepal CO-630 and Biosoft EN600, were found to be more effective. About 66% degradation of TPH was achieved in the presence of the Igepal surfactant, compared to 46% in a control run in the absence of any surfactant.

## EXAMPLE IV

The effect of sludge concentration on TPH biodegradation was investigated using two different sludges at concentrations of 20%, 50% and 90%. Each set of flasks contained the following: (a) 16 ml of nutrient medium and 4 g of sludge; (b) 10 ml of nutrient medium and 10 g of sludge; (c) 20 g of sludge and 2 ml of 10x strength nutrient medium. The flasks (250 ml) were

inoculated with 600 µl of actively growing culture from a cyclone fermenter, and incubated on a rotary shaker (200 rpm) at 25°C for 14 days.

The results are given in Table 4.

**Table 4.**

| Sludge Type | Sludge Concentration % v/v | TPH degradation (% of starting amount) |
|---|---|---|
| Sludge A | (a) 20 | 70 |
| | (b) 50 | 56 |
| | (c) 90 | 36 |
| Sludge B | (a) 20 | 91 |
| | (b) 50 | 81 |
| | (c) 90 | 56 |

It was found that sludge concentration affected the extent of TPH degradation.

## EXAMPLE V

A medium referred to herein as NPK medium was formed by replacing, $KH_2PO_4$ and $Na_2HPO_4$, in the nutrient medium, were replaced with a NPK (nitrogen: phosphorus:potassium) fertilizer (15:30:15) at a rate of 0.8 g/L. All other components in the medium were the same as described before. Experiments were conducted with two different sludges. Erlenmeyer flasks contained 50% v/v NPK medium and 50% v/v sludge together with 0.25% surfactant (Igepal CO-630) based on total culture volume.

Other conditions were the same as those described in Example III. The results are given in Table 5.

**Table 5.**

| Source of Sludge | Medium | TPH degradation (% of starting amount) |
|---|---|---|
| Sludge A | Nutrient | 60 |
| | NPK | 58 |
| Sludge B | Nutrient | 73 |
| | NPK | 71 |

No significant differences were observed between the results obtained with basal medium and with NPK medium.

## EXAMPLE VI

Biodegradation of TPH in different sludges was performed in flasks under shaking conditions. Erlenmeyer flasks containing NPK medium and sludge (50:50, v/v) were inoculated with the actively growing mixed culture, and incubated for 14 days at 30°C.

**Table 6.**

| Sludge Type | Sludge Concentration(%) | TPH degradation (%) |
|---|---|---|
| A | 50 | 61 |
| B | 50 | 76 |
| D | 12.5 | 54 |
| E | 50 | 89 |
| G | 10 | 42 |

The results indicate that 42 to 89% degradation of TPH can be obtained using this process. Sludge G, being a heavy oil sludge, was degraded the least.

## EXAMPLE VII

Alternative complex nitrogen sources to yeast extract were tested using Sludge A and Sludge B. This experiment was carried out using NPK medium 50% v/v, sludge 50% v/v and 0.25% v/v Igepal CO-630 in 250 ml Erlenmeyer flasks incubated at 25°C for 14 days on a rotary shaker (200 rpm).

The results are given in Table 7.

**Table 7.**

| Sludge Type | Complex nitrogen source | TPH degradation (% of starting amount) |
|---|---|---|
| Sludge A | Yeast extract | 59 |
| | Corn steep solids | 52 |
| | Cottonseed protein | 51 |
| | Potato protein | 49 |
| Sludge B | Yeast extract | 75 |
| | Corn steep solids | 85 |
| | Cottonseed protein | 83 |
| | Potato protein | 79 |

All the alternative nitrogen sources tested, at a final culture concentration of 0.5 g/L, gave similar performance to 0.5 g/L yeast extract.

## EXAMPLE VIII

Biodegradation of different hydrocarbon fractions was tested, using Sludge B. Erlenmeyer flasks that contained 50% v/v sludge, 50% NPK medium and 0.25% Igepal CO630. After inoculation with an actively growing culture, flasks were incubated on a rotary shaker for 14 days at 30°C. The whole content of the flask was

extracted once with hexane followed by dichloromethane. After centrifugation both extracts were combined and the solvent evaporated. Residual hydrocarbon was dissolved in hexane and centrifuged. A known weight of hexane soluble portion was passed through a column (0.75 x 27 cm) of silica gel (activated at 100°C overnight). Successive applications of hexane (120 ml), dichloromethane (30 ml) and chloroform: methanol (1:1, 15 ml) produced fractions containing saturated, aromatics and polar (resins) hydrocarbons, respectively.

The results are given in Table 8.

**Table 8.**

| Fraction | % of total hydrocarbons | % degradation |
|---|---|---|
| Saturate | 73-77 | 73-77 |
| Aromatics | 11-13 | 65-69 |
| Resins | 8-10 | 61-63 |

The results indicate that all of the major TPH component were degraded.

## EXAMPLE IX

This experiment was conducted to determine if pretreatment with an advanced oxidative process (Fenton's reagent viz. $H_2O_2$ + $FeSO_4$) could enhance TPH degradation in sludge. Pretreatment and subsequent biodegradation was carried out in the same flask. For pre-treatment, Sludge A was diluted with water to obtain 20 ml of a 50% v/v sludge concentration. pH of the mixture was adjusted to 4.0 by adding 4N HCl. $H_2O_2$ and $FeSO_4$ were added at concentrations of 0.3% v/v and 10 millimolar, respectively.

The flasks were kept on a rotary shaker (200 rpm) at 25°C for 2 days. Thereafter, 2 ml of NPK medium (10 times concentrated) were added in solid form and the pH adjusted to 7.0 by addition of 2N NaOH solution. The flasks were inoculated with an actively growing inoculum (600 µl) from a cyclone fermenter and incubated on a rotary shaker for a period of 28 days. The following treatments were tested: (a) no pre-treatment or addition of surfactant; (b) Fenton's reagent pre-treatment, without surfactant; (c) addition of 0.25% Igepal CO-630, without Fenton's pretreatment; and (d) Fenton's reagent pretreatment in the presence of 0.25% Igepal CO-630.

The results are given in Table 9.

**Table 9.**

| Treatment | Incubation time (days) | | | |
|---|---|---|---|---|
| | 7 | 14 | 21 | 28 |
| | % TPH degradation | | | |
| None | 28 | 42 | 46 | 53 |
| Fenton's pre-treatment (48 h) | 36 | 61 | 64 | 65 |
| Surfactant (0.25 %) | 42 | 61 | 66 | 72 |
| Fenton's pre-treatment (48 h) in the presence of surfactant (0.25 %) | 43 | 64 | 68 | 72 |

The results indicate that pre-treatment of sludges with an oxidative agent or addition of surfactant significantly increased the extent of degradation of TPH in sludge oil.

## EXAMPLE X

The performance of biodegradation of TPH in different sludges was evaluated in different reactor types. The reactors tested were of different configuration and scale. Biodegradation tests in Erlenmeyer flasks were performed as described in other examples. Cyclone fermenters were as described above. Air-lift reactors were fitted with spargers and connected to an air source. The mixing in the reactors was achieved by supplying air at the rate of 0.5 volume/volume/minute and 0.125% surfactant. NPK medium and sludge (50:50 v/v) was used in these experiments. All the reactors were inoculated with an actively growing mixed culture.

The results are presented in Table 10.

**Table 10.**

| Sludge Type | Reactor Type | Scale of Process (litres) | Incubation time (days) | TPH biodegradation (%) |
|---|---|---|---|---|
| E | Erlenmeyer flask | 0.25 | 20 | 74 |
| E | Air-lift | 150 | 14 | 70 |
| F | Air-lift | 150 | 14 | 84 |
| B | Erlenmeyer flask | 0.25 | 14 | 81 |
| B | Cyclone | 1 | 8 | 85 |
| C | Air-lift | 18 000 | 11 | 84 |

The results show that efficient sludge degradation occurs in different aerated reactor types and at different scales of operation ranging from laboratory to production scale.

## EXAMPLE XI

Biodegradation of TPHs in clay fines was evaluated in shake flask cultures. Flasks containing clay fines (TPH, 10.5%, w/v, and NPK medium 50:50, w/v) and 0.25%, w/v Igopal CO-630 were inoculated with an actively growing culture and incubated for 14 days at 30°C. The residual TPH content was determined and results are shown in Table 11.

**Table 11**

| Incubation Time (days) | % TPH degradation |
|---|---|
| 7 | 77 |
| 14 | 92 |

The results indicate that 92% of clay fines can be achieved in 14 days by using this process.

**CLAIMS:**

1. A method for the biodegradation of an oil-based sludge, said oil-based sludge comprising a mixture of petroleum hydrocarbons, said method comprising the steps of:

(a) forming an aqueous solution in a reactor of an oil-in-water emulsion, bacterial culture and nutrients for said bacterial culture,

said oil-in-water emulsion being an emulsion of said oil-based sludge in water,

said bacterial culture having the ability to grow on petroleum hydrocarbons as sole carbon source and having been isolated from a hydrocarbon contaminated soil or hydrocarbon-containing sludge or other environments rich in hydrocarbon degrading bacteria, by microbial enrichment techniques using hydrocarbons in the selection medium,

said reactor containing up to 50% by volume of said hydrocarbons;

(b) maintaining said aqueous solution under aerobic conditions in the reactor at a temperature of at least 10°C for a period of time sufficient to reduce the amount of hydrocarbon by at least 25%, and at a pH conducive for promotion of bacterial growth and hydrocarbon degradation; and

(c) discharging aqueous solution having a reduced amount of said hydrocarbons from said reactor.

2. The method of Claim 1 in which said nutrients comprise chemical components of bacterial cells in proportions corresponding to relative proportions in

naturally-occurring bacterial cells, and supplied at concentrations which promote high levels of bacterial growth and high rates of hydrocarbon degradation.

3. The method of Claim 1 or Claim 2 in which said nutrients comprise bioavailable nitrogen and phosphorous.

4. The method of any one of Claims 1-3 in which the reactor contains about 5-50% by volume of said petroleum hydrocarbons.

5. The method of Claim 4 in which the reactor contains about 10-30% by volume of said petroleum hydrocarbons.

6. The method of any one of Claims 1-5 in which said oil-based sludge contains hexane-extractable hydrocarbons in an amount in the range of up to 500 000 ppm.

7. The method of Claim 6 in which the amount of hexane-extractable hydrocarbons is in the range of 65 000-250 000 ppm.

8. The method of any one of Claims 1-7 in which the petroleum hydrocarbons consist of mixtures of saturated hydrocarbons, aromatic hydrocarbons, hydrocarbon resins and asphaltenes.

9. The method of any one of Claims 1-8 in which the amount of nitrogen is in the range of 50-1000 ppm and the amount of phosphate is in the range of 10-200 ppm.

10. The method of any one of Claims 1-9 in which the nitrogen compound is an ammonium ion, nitrate or organic nitrogen, and the phosphorus is phosphate.

11. The method of any one of Claims 1-10 in which there is a nonionic surfactant in an amount sufficient to form said oil-in-water emulsion.

12. The method of any one of Claims 1-10 in which there is an anionic surfactant in an amount sufficient to form said oil-in-water emulsion.

13. The method of Claim 11 or Claim 12 in which the amount of surfactant is less than 2500 ppm.

14. The method of Claim 11 or Claim 12 in which the amount of surfactant is less than 1500 ppm.

15. The method of any one of Claims 1-14 in which the nutrient contains at least one of magnesium, manganese, sulphate, organic sulphur, calcium, ferric ion, copper.

16. The method of Claim 15 in which the nutrient additionally contains cobalt, zinc, boron, or molybdenum.

17. The method of any one of Claims 1-16 in which the amount of petroleum hydrocarbon is reduced by at least 75%.

18. The method of any one of Claims 1-16 in which the amount of petroleum hydrocarbons is reduced by at least 50%.

19. The method of any one of Claims 1-18 in which said aqueous solution contains a surfactant, the ratio of the amount of petroleum hydrocarbon to surfactant being at least 40:1.

20. The method of any one of Claims 1-19 in which the aqueous solution is maintained in step (b) in at least two reactors in series, prior to being discharged in step (c).

21. The method of any one of Claims 1-20 in which the aqueous solution is maintained in step (b) for a hold-up time of at least 7 days.

22. The method of any one of Claims 1-21 in which the relative composition of the nutrients reflects the known relative composition of required components for growth of bacteria.

23. The method of any one of Claims 1-22 in which a proportion of the degraded sludge is retained in the reactor after discharge as an inoculum for the next batch of sludge.

24. The method of any one of Claims 1-23 in which the reactor is operated as a fed-batch, continuous or semi-continuous system.

25. The method of any one of Claims 1-24 in which the sludge is chemically or physically pretreated to improve biodegradability prior to or during biodegradation.

26. The method of any one of Claims 1-25 in which partial petroleum hydrocarbon degradation occurs resulting in separation of a water and an oil phase, said oil phase being recycled to the next sludge degradation batch.

27. The method of Claim 26 in which the separated oil phase is recovered.

28. The method of Claim 26 in which the separated water phase is rich in hydrocarbon degrading bacteria and is used as a bacterial inoculum to accelerate bioremediation of hydrocarbon contaminated soil.

29. The method of Claim 26 in which the bacteria are recovered from the water phase for subsequent use as a bacterial inoculum.

30. The method of any one of Claims 1-29 in which the sludge contains chlorinated hydrocarbons.

31. The method of Claim 30 in which the sludge contains polychlorinated biphenyls.

32. The method of any one of Claims 1-29 in which the sludge is a byproduct of coal processing.

33. The method of any one of Claims 1-29 in which the sludge is a petroleum refinery sludge.

34. The method of any one of Claims 1-29 in which the sludge is in the form of oil-containing clay fines.

35. The method of any one of Claims 1-29 in which the sludge is obtained from the bottom of a storage tank for oil.

36. The method of any of one Claims 1-29 in which the sludge is a petroleum residue from an on-land well head or from the washing of a hold in a tanker.

37. The method of any one of Claims 1029 in which the petroleum residue comprises slop oil or treater emulsions.

38. The method of any one of Claims 1-37 in which the sludge is admixed with organic molecules other than said petroleum hydrocarbons.

39. The method of Claim 38 in which the organic molecules are degraded in step (b).

40. The method of Claim 38 in which the organic molecules are not degraded in step (b).

41. The method of any one of Claims 1-40 in which said bacterial culture is indigenous bacteria in the oil-based sludge, such indigenous bacteria multiplying and degrading the sludge.

42. The method of any one of Claims 1-40 in which the inoculum comprises one or more hydrocarbon degrading

microbial strains produced by fermentation under sterile conditions.

43. The method of any one of Claims 1-40 in which the temperature in step (b) is in the range of 20-37°C.

44. The method of any one of Claims 1-40 in which the temperature in step (b) is in the range of 27-33°C.

45. The method of any one of Claims 1-29 in which the oil-based sludge is admixed with soil.

# INTERNATIONAL SEARCH REPORT

In ational Application No
PCT/CA 98/00108

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C02F11/00 C02F11/02 C02F3/12 C02F3/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C02F

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | PATENT ABSTRACTS OF JAPAN<br>vol. 097, no. 006, 30 June 1997<br>& JP 09 038630 A (CORONA GIKEN KOGYO KK;KUNO TADAHIKO), 10 February 1997,<br>see abstract<br>-& DATABASE WPI<br>Section Ch, Week 9716<br>Derwent Publications Ltd., London, GB;<br>Class D15, AN 97-173824<br>XP002065438<br>see abstract | 1,11,12, 25 |
| X | US 5 271 845 A (PAQUIN JEAN) 21 December 1993<br>see column 1, line 59 - column 2, line 3<br>see column 5, line 4 - line 18; claim 10 | 1,4,22, 23,43,44 |

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 19 May 1998 | 04/06/1998 |

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document. with indication.where appropriate. of the relevant passages | Relevant to claim No. |
|---|---|---|
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| A | PATENT ABSTRACTS OF JAPAN<br>vol. 017, no. 561 (C-1119), 8 October 1993<br>& JP 05 161900 A (FUJITA CORP), 29 June 1993,<br>see abstract | 1,11,12, 23 |

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International Application No
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| | | JP 1034380 A | 03-02-1989 |